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The relationship between second-trimester amniotic fluid CRP levels and pregnancy outcomes

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Abstract

Aim: The aim of this study is to investigate the relationship between CRP levels studied in second-trimester amniotic fluid and pregnancy outcomes.

Material and Methods: Seventy-three single pregnant women who underwent amniocentesis for genetic purposes in our perinatology clinic between 16-22 weeks of gestation and gave birth in our hospital were included in the study. Pregnancies resulting in preterm birth (PTB), preeclampsia, preterm premature rupture of membranes (PPROM), gestational diabetes mellitus (GDM) and postterm pregnancies were defined as "composite outcomes" and these patients were evaluated as Group 1. In addition, patients in Group 1 were divided into five subgroups: PTB, preeclampsia, GDM, PEMR, postterm pregnancies. On the other hand, pregnant women with normal pregnancy outcomes were included in Group 2. All recorded data of these groups were compared.

Results: Amniotic fluid CRP level was 0.10 ± 0.18 mg / L in Group 1 and 0.07 ± 0.08 mg / L in Group 2 (p: 0.94). In Group 2, gestational age and fetal birth weight were higher. However, this difference was not significant. When CRP values in individual subgroups were compared in terms of composite results (preeclampsia, PTB, GDM, PPROM and postterm pregnancy), amniotic fluid CRP levels were higher than in the general population.

Discussion: Our study presented evidence that second-trimester amniotic fluid CRP level is not associated with PD, PPROM, preeclampsia, postterm pregnancy and GDM, which we consider as composite obstetric outcomes.

Keywords

Amniotic Fluid, CRP, Pregnancy, Composite Outcomes, Second Trimester

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Introduction

Predicting poor obstetric outcomes in pregnancies is one of the main goals of prenatal follow-up. Preterm birth (PTB), which accounts for 70% of perinatal mortality and approximately 50% of long-term neurological damage, is one of the most important problems in obstetrics [1, 2]. Preeclampsia is among the important complications affecting approximately 2-8% of pregnancies. Predicting poor obstetric outcomes of pregnancies could improve maternal and fetal outcomes.

Many supportive parameters, such as clinical and biochemical markers or Doppler ultrasound, have been reported to help predict and manage poor obstetric outcomes [3]. Among these, biochemical markers of first and second-trimester screening tests (alpha fetoprotein, beta-human chorionic gonadotropin, pregnancy-associated placental protein A, estriol), maternal serum IL-6, C-reactive protein (CRP) levels and amniotic fluid (usually after early membrane rupture), acute inflammatory markers that are evaluated [4, 5].

CRP is an inflammatory marker found in maternal serum [6]. There are studies that establish a relationship between increased maternal serum CRP levels and increased PTB or preeclampsia [7]. There are also publications evaluating the prediction of PTB by CRP levels in amniotic fluid [8]. Vecchie et al., suggested in their recent studies that maternal serum CRP levels may be an effective and appropriate tool to identify pregnant women at high risk for maternal and fetal complications [9].

The primary aim of the study was to investigate the relationship between second-trimester amniotic fluid CRP level and pregnancy outcomes, defined as composite outcomes. The secondary aim was to compare amniotic fluid CRP levels with the levels of PTB, preeclampsia, PPROM, GDM and postterm pregnancies separately.

Material and Methods

Seventy-three patients who underwent amniocentesis at Şanlıurfa Training and Perinatology Research Hospital from January to December 2019 were included in the study. Singleton pregnant women at 16-22 weeks of gestation who underwent genetic amniocentesis and gave birth in our hospital were included in the study. All patients had uncomplicated health conditions before amniocentesis. There is no fetal major property. Women whose medical information and pregnancy results could not be obtained (who did not give birth in our hospital), women with chronic diseases (such as kidney, cardiovascular, liver or autoimmune diseases), vaginal infections, multiple pregnancies, patients who developed complications due to amniocentesis (abortion within the first 10 days or amniotic fluid leakage), patients with a history of PTB, late abortion, late pregnancy complications in their previous pregnancies, and patients with infection (such as urinary tract infection) were excluded from the study.

A total of 73 patients who met the criteria were included in the study. Pregnancies resulting in PTB, preeclampsia, preterm premature rupture of membranes (PPROM), gestational diabetes mellitus (GDM) and postterm pregnancy were defined as "composite outcomes" and these patients were assigned to Group 1. In addition, patients in Group 1 were divided into five subgroups: PTB, preeclampsia, GDM, PEMR, postterm pregnancy. On the other hand, pregnant women with normal pregnancy outcomes were included in Group 2.

Genetic amniocentesis is performed between 16-22 weeks in our clinic. The procedure is performed by using a 22 gauge spinal needle via the transabdominal route and using a free-hand technique accompanied by real-time ultrasonography. The first 0.5 cc of fluid taken during amniocentesis is discarded due to the risk of maternal contamination. Then, 2 cc of amniotic fluid taken was stored in the storage unit in our clinic at -20 °C for 1 year. In case of need in the future, the storage process is routinely applied to each patient, so that the patient does not need to perform an invasive procedure again. After this sample, 1 ml of amniotic fluid is taken per week for genetic analysis. Patients with stored two-milliliter samples are routinely disposed of after giving birth. The main indications for the genetic amniocentesis procedure in our service are advanced maternal age, abnormal maternal serum screening results and abnormal ultrasound findings, and other reasons (such as a history of a child with chromosomal anomaly, maternal anxiety). Advanced maternal age was defined as the mother's age at birth over 35 years. Abnormal maternal serum screening test includes pregnant women with high risk for trisomy 18 and 21 (calculated combined risk ≥1/340 for trisomy 21, ≥1/100 for trisomy 18). We decide abnormal ultrasound criteria are increased nuchal fold, choroid plexus cyst, pyelectasis, single umbilical artery, early weekday oligohydramnios, early week intrauterine growth retardation, early week polyhydramnios, hyperechogenic bowel and hyperechogenic cardiac focus. The family program for chromosomal abnormalities is comprehensive and completes the family related to aneuploidy, translocation and inversion chromosomes. Patients with maternal anxiety but whose maternal serum screening results were below the determined threshold value (in the low-risk group) were named as amniocentesis upon the request of the family and were kept under the heading of other causes.

For the diagnosis of preeclampsia, a systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg after the 20th week of pregnancy was defined as hypertension measured at least twice at 4-hour intervals in the left lateral decubitus position. In addition, the 24-hour urine proteinuria level of a pregnant woman without kidney disease was defined as >300 mg [10]. Preterm birth was diagnosed as delivery before 37 weeks. PPROM was diagnosed as a rupture of fetal membranes before the onset of uterine contractions before 37 weeks. The diagnosis of GDM was made by screening all patients with a 75 g oral glucose test between 24-28 weeks of gestation. Postterm pregnancy was defined in pregnant women whose last menstrual period and first-trimester ultrasound exceeded 41 weeks, but labor had not yet started.

Ethics Committee

Harran University Clinical Research Ethics Committee approved the study (decision number 20.09.04, dated 11.05.2020). The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 20; SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation or percentiles. The distribution of

data was determined using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk's test). The Mann-Whitney U-test was used for non-parametric numerical data and Student's t-test was used for parametric numerical data. Categorical data were compared using chi-square or Fisher's exact test. A p-value less than 0.05 was considered significant.

Results

During the study, a total of 58,983 live births were performed in our hospital. In the same period, amniocentesis was performed in 565 patients in our clinic. Of these cases, 486 were genetically motivated amniocentesis. Seventy-three patients who met all inclusion criteria were included in the study. Of these patients, 32 (43.8%) had composite results (Group 1) and 41 (56.2%) had uncomplicated pregnancy outcomes (Group 2).

Demographic characteristics and amniotic fluid CRP level data are given in Table 1. Amniotic fluid CRP level was 0.10 ± 0.18 mg/L in Group 1 and 0.07 ± 0.08 mg/L in Group 2 (p: 0.94). There was no significant difference between the demographic characteristics and CRP levels of the two groups. Table 2 summarizes the maternal and fetal outcomes of the two groups. Gestational age at birth and newborn birth weight were higher in Group 2. Nine (28.1%) patients in Group 1 and 5 (12.2%) patients in Group 2 delivered by cesarean section (p: 0.086). In Group 1, the need for neonatal intensive care was significantly higher (p: 0.036).

When the CRP values of the individual subgroups were compared according to the composite results (preeclampsia, PTB, GDM, PPROM, and postterm pregnancy), no significant difference was observed between the amniotic fluid CRP levels (Table 3).

Table 1. Demographic characteristics and amniotic fluid CRP levels of the two groups.

	Total n: 73	Group 1 n: 32	Group 2 n: 41	p value
Maternal age (year)	27.06±6.56	27.12±6.64	27.02±6.57	0.97
Body mass index	29.4±1.88	29.24±1.97	29.07±1.82	0.71
Gravity	3.82±2.78	3.87±2.95	3.78±2.66	0.88
Parity	2.26±2.13	2.18±2.20	2.31±2.10	0.75
Smoking (%)				
Yes	34 (46.6)	14 (43.8)	20 (48.8)	0.66
No	39 (53.4)	18 (56.3)	21 (51.2)	
Week of gestation at which amniocentesis was performed	19.39±1.99	19.37±1.75	19.41±2.19	0.92
Transplacental passage during the procedure (%)				
Yes	19 (26)	8 (25)	11 (26.8)	0.86
No	54 (74)	24 (75)	30 (73.2)	
Amniotic fluid CRP level (mg/L)	0.08±0.13	0.10±0.18	0.07±0.08	0.94

Values are expressed as mean ± SD or numbers and percentages (%). p <0.05 is considered statistically significant.

Table 2. Comparison of obstetric and fetal outcomes of the two groups.

	Total n: 73	Group 1 n: 32	Group 2 n: 41	p
Gestational week at birth	36.87±2.35	35.50±2.96	37.95±0.66	<0.001
Birth type (%)				
Vaginal birth	59 (80.8)	23 (71.9)	36 (87.8)	0.086
Cesarean delivery	14 (19.2)	9 (28.1)	5 (12.2)	
Birth weight (gr)	3182±579.63	2882.68±693.77	3417.07±321.57	<0.001
Need for NICU				
Yes	11 (15.1)	8 (25)	3 (7.3)	0.036
No	62 (84.9)	24 (75)	38 (92.7)	

Values are expressed as mean ± SD or numbers and percentages (%),
NICU: Neonatal intensive care unit. p <0.05 is considered statistically significant.

Table 3. Relationship between pregnancy outcomes and amniotic fluid CRP levels.

	Amniotic fluid CRP value	p
Preterm birth (25)	0.089±0.16	0.591
Term birth (48)	0.085±0.121	
Preeclampsia		
Yes (2)	0.025±0.035	0.395
None (71)	0.088±0.138	
GDM		
Yes (2)	0.105±0.91	0.455
None (71)	0.080±0.13	
PPROM		
Yes (3)	0.320±0.35	0.162
None (70)	0.076±0.11	
Postterm pregnancy		
Yes (3)	0.27±0.35	0.235
None (70)	0.78±0.119	

Values are expressed as mean ± SD or numbers and percentages (%).
GDM; gestational diabetes mellitus, PPROM; preterm premature rupture of membranes.
p <0.05 is considered statistically significant.

Discussion

CRP is an acute phase protein synthesized by liver cells in response to proinflammatory cytokines [6, 11]. It has been suggested that endothelial dysfunction is an exaggerated maternal inflammatory response to pregnancy. Yudkin et al. stated that CRP is strongly associated with endothelial activation and dysfunction markers [12]. Therefore, the theory is becoming more common that the fetus is connected by an intrauterine inflammatory process in the very early weeks of pregnancy, increasing the subclinical inflammatory response. Indeed, it is known that inflammation develops during implantation and then decreases towards the middle of pregnancy due to maternal tolerance to fetal antigens [9, 13]. Inflammatory markers that rise in the second trimester may be early signs of the development of maternal and fetal complications. In our study, the amniotic fluid CRP levels of those with composite results and the diagnosis of PTB, PPROM and GDM in individual subgroups were higher than in the general population. However, this difference was not significant.

PTB is one of the most important causes of fetal mortality and morbidity all over the world. Therefore, maternal serum and amniotic fluid biomarkers in the prediction of PTB are among the most studied subjects recently. Hallingström et al. found no significant difference between spontaneous PTB and term delivery in terms of second-trimester amniotic fluid CRP values [14]. In another similar study, it was shown that second-trimester amniotic fluid CRP level was not associated

with PTB [5]. Borna et al. showed that the amniotic fluid CRP levels of pregnant women who had PTB had no predictive value [1]. Koçyiğit et al. found higher CRP levels in pregnant women who had PTB, but they showed that this difference was not statistically significant [15].

Pregnant women with PPROM may be complicated by adverse conditions such as microbial invasion of the amniotic cavity (MIAC) and intraamniotic inflammation (IAI). There is also a publication showing that serum CRP concentrations are significantly increased in PPROM patients with MIAC or IAI [16]. CRP samples in the amniotic fluid were obtained by amniocentesis performed approximately five hours after the diagnosis of PPROM between 31-34 weeks. In our study, CRP level was studied in samples taken from second trimester and asymptomatic pregnant women. In our study, amniotic fluid CRP levels in pregnant women with PPROM (0.320±0.35 mg/L) were higher than in those without PPROM (0.076±0.11 mg/L). However, this difference is not statistically significant. On the other hand, the number of PPROM pregnant women in our study is limited to 3 patients.

GDM, which is a common metabolic disorder in pregnancy, is a serious risk for maternal and neonatal adverse outcomes such as polyhydramnios, fetal macrosomia, shoulder dystocia. Early diagnosis and timely treatment are beneficial in reducing medical complications and reducing societal costs. Publications showing the relationship between CRP level and GDM are contradictory [17]. A recent review showed that serum CRP levels are significantly elevated in pregnant women with GDM compared to healthy pregnant women. Therefore, CRP has been considered a new marker in the diagnosis of GDM. In the literature, CRP was measured from only one postpartum placental sample [4], and in all other studies, CRP was measured from serum samples [17]. We could not find any study investigating the relationship between CRP levels in amniotic fluid and GDM. In our study, second-trimester amniotic fluid CRP levels of patients with GDM were higher (0.105±0.91 mg/L) compared to pregnant women who were not diagnosed with GDM (0.08±0.13 mg/L). However, this difference is not statistically significant. In our study, the number of GDM cases is not sufficient to show this relationship.

Due to the role of CRP in inflammation, it is considered that CRP can also be related with the inflammation response in preeclampsia. Therefore, studies have led to the idea of controlling the levels of CRP in order to determine the predictive value in preeclampsia clinics [7, 18]. Studies conducted in recent years have mostly focused on the relationship between serum CRP levels and the severity of preeclampsia [19]. Studies show that preeclampsia is characterized by the exaggeratedly increased maternal systemic inflammatory response. We did not find any publications that associate second-trimester amniotic fluid CRP levels with preeclamptic pregnant women. There were two preeclampsia patients in the cohort in our study. We did not observe a significant difference between the amniotic fluid CRP level in these two patients and in patients without preeclampsia.

Our study has some limitations. First, amniocentesis is an invasive approach to predict adverse pregnancy outcomes and complications, and it does not seem possible to be routinely applied in daily practice. In our study, we examined CRP from only one sample in the second trimester, that is why we were unable to evaluate CRP level differences, which can fluctuate till birth. On the other hand, the number of our patients was limited. Therefore, in our study, the number of patients with adverse obstetric outcomes, although cumulatively relatively sufficient, was limited when evaluated separately.

Our study presented evidence that second-trimester amniotic fluid CRP level is not associated with PTB, PPROM, preeclampsia, postterm pregnancy and GDM, which we consider as composite obstetric outcomes.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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